VOLUME 29 AUGUST 1951 NUMBER 4

Canadian Journal of Zoology

Editor: E. HORNE CRAIGIE

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF ZOOLOGY

This was formerly *Section D, Canadian Journal of Research.* The change to the new name took place January 1, 1951. The CANADIAN JOURNAL OF ZOOLOGY is published six times annually.

The CANADIAN JOURNAL OF ZOOLOGY is published by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. Matters of general policy are the responsibility of a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

The National Research Council of Canada publishes also the *Canadian Journal of Botany, Canadian Journal of Chemistry, Canadian Journal of Medical Sciences, Canadian Journal of Physics, Canadian Journal of Technology.*

Subscription rate: $2.00 a year. All enquiries concerning subscriptions should be addressed to the CANADIAN JOURNAL OF ZOOLOGY, National Research Council, Ottawa, Canada. Special rates can be obtained for subscriptions to more than one of the Journals published by the National Research Council.

Canadian Journal of Zoology

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOLUME 29 AUGUST, 1951 NUMBER 4

THE EFFECT OF GONADAL HORMONES ON THE NUCLEIC ACID CONTENT OF LIVER AND SERUM IN THE IMMATURE PULLET, AND THE DIFFERENCE BETWEEN THE NUCLEIC ACID CONTENT OF THE LIVERS OF SEXUALLY MATURE PULLETS AND COCKERELS[1]

BY R. H. COMMON,[2] D. G. CHAPMAN,[3] AND W. A. MAW[4]

Abstract

Treatment of sexually immature pullets with testosterone propionate so as to evoke changes in combs and wattles similar to normal puberal changes did not affect liver weight or liver content of nucleic acids. Estradiol benzoate, or estradiol benzoate plus testosterone propionate, increased liver weight and total liver pentose nucleic acid, and slightly increased liver desoxypentose nucleic acid. Chemical evidence is adduced in support of the view that the increase of liver crude protein caused by estrogen is a consequence of cellular hypertrophy accompanied by a slight degree of hyperplasia. The ratio RNAP : DNAP was relatively high in the liver of the young chicken, but declined somewhat during the first 12 weeks. With the onset of reproductive activity, the ratio RNAP : DNAP increased in the livers of the females but did not show a similar tendency in the males. The results suggest that there is a sexual differentiation in the nucleic acid content of the livers of the mature fowl, and that this is reasonably attributable to endogenous estrogen activity. Data are presented in confirmation of the reported effect of estrogen in increasing serum or plasma nucleic acid in the fowl, androgen being without any such effect.

Introduction

Treatment of mildly androgenized immature pullets with estrogen increases the total liver ribonucleic acid (RNA), the total liver desoxyribonucleic acid (DNA), and the ratio RNA : DNA in the liver (5). In this connection the terms ribonucleic acid and desoxyribonucleic acid were used in their conventional sense, i.e., without implying that the sugar components present were ribose and desoxyribose. The designations pentose nucleic acid and desoxypentose nucleic acid are less objectionable, however, and have been adopted for the purposes of the present paper.

The observations in question (5) were restricted to a comparison of the effects of estrogen plus androgen with those of androgen alone. The reasons for this were that (*a*) the number of pullets which could be properly handled

[1] *Manuscript received March 9, 1951.*

Contribution from the Faculty of Agriculture of McGill University, Macdonald College, P.Q., and supported by a Grant-in-Aid of Research to the senior author from the National Research Council of Canada.

[2] *Professor of Agricultural Chemistry.*

[3] *Research Assistant. Present address: Food and Drug Division, Department of National Health and Welfare, Ottawa.*

[4] *Professor of Poultry Husbandry.*

[The June issue of this Journal (Can. J. Zool. 29 : 167-263. 1951) was issued July 4, 1951.]

was limited and that (*b*) estrogen plus androgen evokes a closer simulation of the normal puberal changes in the pullet than does estrogen alone (10). Androgen by itself is capable of producing a response of the oviduct in the chick (16, 17), but the response is small compared with the effect of estrogen and has sometimes been regarded as relatively negligible (16). The effects of small doses of androgen in augmenting the effect of estrogen on the oviduct are, by contrast, considerable (9, 10). However, in view of the fact that androgen does have a definite, though relatively very slight, effect on the chick oviduct, it is more correct to regard this as a true synergetic effect rather than as an augmenting effect of androgen on the effect of estrogen. Parkes and Emmens (25) considered that the endogenous androgen of the puberal pullet originates principally from the ovary. This view has received more direct support from the recent histological observations of Taber (29). It seems unlikely that androgen of adrenal origin has an importance in this connection comparable with that of androgen of ovarian origin.

The present paper deals with an investigation of the effects of androgen and estrogen on liver nucleic acids when administered separately and together, and with observations directed to the detection of similar changes in the normal puberal pullet.

Experimental

Experiment 1. Effects of Gonadal Hormones on Liver Nucleic Acids

Two groups each of 16 immature cross bred pullets (New Hampshire ♂ × Barred Plymouth Rock ♀) were used. The birds in each group were taken from the same hatching, and both groups were reared on the same diets and under similar conditions until placed under experimental conditions.

The pullets in each group were placed at random in individual laying cages and were assigned at random to four different treatments as indicated in Table I. The amounts of food eaten by the different birds in each group were kept as nearly equal as possible. Such control of food consumption is of fundamental importance in experiments of this kind. The reasons for this have been outlined previously (5).

The administration of hormonal treatments, the methods of slaughter and preparation of samples and the main analytical methods used have been described previously (5). In the experiments now reported estradiol benzoate (Schering) was used instead of estradiol dipropionate.

Liver total lipid was determined by disintegrating a 5 gm. or 10 gm. sample of liver in the Waring blendor with 100 ml. 10% trichloroacetic acid and some "Celite" filter aid. The precipitate was filtered off, dehydrated with ethanol and then thoroughly extracted by refluxing with three changes of alcohol – ethyl ether (3 : 1 by volume). The combined ethanol and ethanol – ether extracts were evaporated under reduced pressure. The lipid was re-extracted from the residue with dried ethyl ether, filtered clear, and weighed after removal of the solvent under reduced pressure. Serum lipid was similarly determined using 5 ml. or 10 ml. samples.

*Experimental Results**

In the first group the pullets were 91 days old at the beginning of the experiment and 103 days old when killed. In the second group the pullets were 80 days old at the beginning and 92 days old when killed. In neither group was there the slightest indication of the onset of normal puberal changes. In the interests of brevity, therefore, the average results for the entire 32 birds are presented together in Tables I and II.

TABLE I

EFFECTS OF ANDROGEN AND ESTROGEN ON OVARIES, OVIDUCTS, LIVER, AND BLOOD OF IMMATURE PULLETS (AVERAGE VALUES)

No. of pullets treated	8	8	8	8
Total dosage of testosterone propionate, mgm.	Nil	6 × 1.0	Nil	6 × 1.0
Total dosage of estradiol benzoate, mgm.	Nil	Nil	6 × 3.0	6 × 3.0
Initial live weight, kgm.	1.01	1.03	1.01	1.03
Final live weight, kgm.	1.22	1.24	1.22	1.23
Food consumption, kgm.	0.92	0.91	0.92	0.92
Ovary, gm.	0.30	0.24	0.26	0.25
Oviduct, gm.	0.16	0.14	9.80	13.1
Liver weight, gm.	20.4	21.4	32.4	32.1
Liver, gm. per kgm. live weight	16.8	17.3	26.6	26.1
Liver crude protein, %	20.5	20.9	19.4	19.3
Liver crude protein, gm. per kgm. live weight	3.45	3.60	5.12	5.05
Serum total lipid, %	0.27	0.27	13.1	11.9
Haematocrit value, %	31.0	35.6	20.2	22.3

It will be noted (Table I) that the hormonal treatments did not affect the live weights. Total food consumption was maintained equal as between the different pullets to a satisfactory degree.

Estrogen alone and estrogen plus androgen produced the usual large degree of hypertrophy of the oviduct and the synergetic effect of estrogen and androgen was in evidence. These two treatments also produced large and similar increases in liver weight and in gm. liver per kgm. live weight. This effect of estrogen is now well established (7, 8). Clavert (7), working with pigeons, has shown that the increase in liver weight produced by estrogen is accompanied by both hypertrophy and hyperplasia of the liver cells. More recently Clavert and Randavel (8) have demonstrated histologically that similar changes take place in the liver of the pigeon during the normal reproductive cycle, presumably in relation to the normal cyclic fluctuations of endogenous estrogen.

The data for serumlipid exemplify the enormous magnitude of the increases which can be evoked in the fowl by estrogen (9, 12, 19). This increase

* *The results secured in this experiment in regard to liver iron have been reported in a separate communication* (4.)

TABLE II

EFFECTS OF ANDROGEN AND ESTROGEN ON LIVER NUCLEIC ACIDS OF THE IMMATURE PULLET. AVERAGES FROM SAME BIRDS AS IN TABLE I

No. of pullets treated	8	8	8	8	Least significant difference ($P = 0.05$)
Total dosage testosterone propionate, mgm.	Nil	6 × 1.0	Nil	6 × 1.0	—
Total dosage, estradiol benzoate, mgm.	Nil	Nil	6 × 3.0	6 × 3.0	—
RNAP, mgm. per 100 gm. fresh liver	78.5	76.4	79.7	85.6	22.96
RNAP, mgm. per liver	16.0	16.3	25.8	27.6	
Liver RNAP, mgm. per kgm. live wt.	13.2	13.2	21.1	22.4	2.06
DNAP, mgm. per 100 gm. fresh liver	36.1	35.9	26.5	27.0	4.29
DNAP, mgm. per liver	7.4	7.6	8.6	8.7	
Liver DNAP, mgm. per kgm. live wt.	6.1	6.3	7.0	7.1	0.84
Average RNAP, mgm. per 100 gm. fresh liver / Average DNAP, mgm. per 100 gm. fresh liver	2.18	2.13	3.00	3.21	
*RNA nitrogen as per cent total liver nitrogen	4.41	4.23	4.72	4.95	0.365
*DNA nitrogen as per cent total liver nitrogen	1.97	1.92	1.47	1.52	0.077
*Nucleic acid nitrogen as per cent total liver nitrogen	6.38	6.15	6.19	6.47	

* *Calculated from values for liver crude protein (averages in Table II) and assuming an N : P ratio of 1.65 for desoxypentose nucleic acid and of 1.69 for pentose nucleic acid (14).*

in large measure represents an increase in phospholipid and is associated with the well known concomitant increase in serum phosphoprotein which is evoked by estrogen. Recent studies have established that the increase in serum phospholipid is mainly in the lecithin–kephalin fraction, the sphingomyelin fraction being relatively unaffected (26).

The data for hematocrit values confirm previous observations (9) that estrogen tends to depress the percentage of red cells while androgen tends to increase the percentage of red cells. This effect of estrogen is doubtless the reason for the fact that reproductive activity tends to increase the sodium content of the blood of turkeys (22), and that estrogen lowers the blood hemoglobin of turkeys (30) and may account for other recorded changes in blood composition (15).

The data for liver nucleic acids demonstrate clearly that, at the level administered, testosterone propionate by itself had not any significant effect upon either the concentrations or the amounts of either RNAP or DNAP in the liver. Estradiol benzoate greatly increased the total amount of liver RNAP and slightly increased the amount of liver DNAP, both effects attaining significance. It will be noted that the concentration of DNAP in the liver was decreased by estradiol benzoate, but that the great increase in liver weight induced by the estrogen resulted in an increase in total liver DNAP.

It may be concluded, therefore, that estrogen greatly increases liver RNAP and also increases liver DNAP, but to a considerably lesser degree. The net result is an increase in the ratio RNAP : DNAP. It may further be concluded that androgen treatments designed to produce effects simulating normal puberal changes of comb and wattles do not appreciably modify these effects of estrogen on liver nucleic acids, although such androgen treatments augment the effects of estrogen on the oviduct to a considerable extent.

The data for liver nucleic acid P and liver crude protein presented in Tables I and II can be used as a measure of the proportion of the liver total nitrogen which is accounted for by nucleic acid nitrogen. In making these calculations the N : P ratios for the nucleic acids must be assumed pending the detailed examination of the liver nucleic acids of the fowl by methods such as those used by Chargaff *et al.* (6). However, the values for the percentages of pentose nucleic acid nitrogen (RNAN) and desoxypentose nucleic acid nitrogen (DNAN) in the liver total nitrogen as calculated in Table II are probably not seriously in error.

It will be noted that estrogen increased the percentage RNAN, though not to a significant degree, whereas estrogen plus androgen give a highly significant increase. The percentage DNAN was not affected by androgen, but was highly significantly reduced by estrogen and by estrogen plus androgen.

The results demonstrate that the increase in liver RNAP : DNAP previously reported (5) was a consequence of estrogen treatment, and fully support the suggested association of this increase with the stimulation of the phosphoprotein synthesizing function of the liver by estrogen. Androgen is known not to stimulate this function to any appreciable extent (9), at any rate when administered at the levels used in the present experiment, and, as might be expected in view of this fact, androgen did not affect the ratio RNAP: DNAP.

The results also confirm the view that the increase of liver crude protein as a result of estrogen treatment is, for the greater part, due to cellular hypertrophy. This is shown by the fact that the RNAP underwent by far the greater increase. It may reasonably be concluded that cellular hyperplasia plays a minor part in contributing to the increase of total liver crude protein. These chemical observations are, therefore, in accord with the histological observations of Clavert and Randavel (8) and of Clavert (7).

Any interpretation of the data for liver DNAP, in terms of the composition of individual cells, must take cognizance of the observation that estrogen increases the proportion of binucleate cells present in the liver of the rabbit (1). Such cells are normally present in the rabbit's liver. The authors are not aware, however, of any references to their occurrence in the fowl's liver, although Clavert and Randavel (8) remarked upon an increased number of mitoses in the liver of estrogenized pigeons.

If the possible effect of estrogen in bringing about an increased proportion of binucleate cells be neglected in the first instance, then the data of Table II

may be used to calculate (*a*) the number of liver cells per kgm. live weight from the DNAP content, assuming constancy of the amount of DNAP per somatic cell; and (*b*) the amount of RNAP per cell. The basis taken for this calculation is the figure of 2.39×10^{-9} mgm. DNA per hepatic cell as determined by Mirsky and Ris (23). Assuming a phosphorus content of 9.4% for the DNA of the fowl's liver, this corresponds to 0.225×10^{-9} mgm. DNAP per hepatic cell. The relevant data and calculated figures are set out in Table III.

TABLE III

EFFECT OF ANDROGEN AND ESTROGEN ON CALCULATED NUMBER OF LIVER CELLS PER KGM. LIVE WEIGHT AND ON PENTOSE NUCLEIC ACID CONTENT OF THE LIVER CELL

No. of pullets treated	8	8	8	8
Total dosage testosterone propionate, mgm.	Nil	6 × 1.0	Nil	6 × 1.0
Total dosage estradiol benzoate, mgm.	Nil	Nil	6 × 3.0	6 × 3.0
Liver DNAP, mgm. per kgm. live weight	6.1	6.3	7.0	7.1
Calculated no. of liver cells per kgm. live weight	2.71×10^{10}	2.80×10^{10}	3.12×10^{10}	3.15×10^{10}
Liver RNAP, mgm. per kgm. live weight	13.2	13.2	21.1	22.4
Calculated RNAP, mgm. per liver cell	0.49×10^{-9}	0.47×10^{-9}	0.65×10^{-9}	0.71×10^{-9}
DNAP, mgm. per liver cell (assumed constant)	0.225×10^{-9}	0.225×10^{-9}	0.225×10^{-9}	0.225×10^{-9}

Similar calculations have been made for the results previously reported for similar experiments (5). The figures are set out in Table IV for comparison

TABLE IV

EFFECT OF ESTROGEN TREATMENTS SUPERIMPOSED ON ANDROGEN TREATMENT ON NUMBER OF LIVER CELLS PER KGM. LIVE WEIGHT AND ON PENTOSE NUCLEIC ACID CONTENT OF LIVER CELLS. CALCULATED FROM RESULTS PREVIOUSLY REPORTED (4)

No. of pullets treated	6	6	6	6
Total dosage testosterone propionate, mgm.	6 × 0.75	6 × 0.75	6 × 0.75	6 × 0.75
Total dosage estradiol dipropionate, mgm.	Nil	6 × 1.0	6 × 2.0	6 × 4.0
Liver DNAP, mgm. per kgm. live weight	5.83	6.73	6.35	6.40
Calculated no. of liver cells per kgm. live weight	2.6×10^{10}	3.0×10^{10}	2.8×10^{10}	2.9×10^{10}
Liver RNAP, mgm. per kgm. live weight	10.0	13.8	15.2	16.1
Calculated RNAP, mgm. per liver cell	0.39×10^{-9}	0.46×10^{-9}	0.54×10^{-9}	0.59×10^{-9}

with the figures in Table III. In both experiments estrogen increased the calculated number of hepatic cells, but testosterone propionate by itself was without any such effect. The results of these calculations afford a more direct estimate of the slight degree of hyperplasia which appears to be induced by estrogen, subject always to the reservation that the degree of hyperplasia may be exaggerated through neglect of the influence of a possible increased proportion of binucleate cells in the livers of the estrogenized pullets.

Effect of Gonadal Hormones on Serum Nucleic Acids

Information as to the nucleic acid content of blood serum is not very extensive. Corradetti (11) reported that blood platelets give a negative Feulgen reaction, but was careful to point out that his results merely showed that DNA, if present, was present in amounts too small to be detected by the test. Leyva (18) found that human serum gave a positive diphenylamine reaction for DNA and stated that the intensity of the reaction varied with the severity of tuberculosis in the patients from whom the samples were drawn.

More recently Mandel *et al.* (20) have studied the effects of estradiol dipropionate upon the RNAP and DNAP of the plasma of the duck. These workers employed the phosphorus partition method of Schmidt and Thannhauser (27) for their analyses. Their ducks were given daily doses of 1 mgm. estradiol dipropionate by injection for periods of from 6 to 10 days. These workers found that the RNAP of the plasma of control birds averaged about 3.3 mgm. per 1000 ml. and that the level rose to an average of about 22 mgm. per 1000 ml. in the estrogenized birds. They found traces of DNAP in the plasma of the controls and slightly more in the estrogenized birds.

In the case of one of the groups of birds in Experiment 1, it was decided to repeat these observations on the sera of the pullets used. At the time of slaughter the blood was collected with precautions against haemolysis and allowed to clot spontaneously. Five ml. portions of the sera were precipitated with 10% trichloroacetic acid. The precipitate was dehydrated with ethanol, thoroughly extracted with ethyl ether alcohol (1 : 3), freed from solvent under reduced pressure, and analyzed for RNAP and DNAP by the phosphorus partition method of Schmidt and Thannhauser (27), the actual phosphorus estimations being carried out by the method of Berenblum and Chain (2). The results are set out in Table V.

TABLE V

EFFECT OF ANDROGEN AND ESTROGEN ON THE SERUM NUCLEIC ACIDS OF THE IMMATURE PULLET

No. of pullets treated	4	4	4	4
Total dosage testosterone propionate, mgm.	Nil	6 × 1.0	Nil	6 × 1.0
Total dosage estradiol benzoate, mgm.	Nil	Nil	6 × 3.0	6 × 3.0
Total nucleic acid P, mgm. per 100 ml. serum	0.45	0.49	3.08	3.71

The value of 0.45 mgm. nucleic acid P per 100 ml. serum of unestrogenized birds is slightly higher than that reported by Mandel *et al.* (20) for ducks, but is of the same order as the values reported by Mandel and Métais (21) for human sera. The values for the estrogenized pullets are somewhat higher than those reported by Mandel *et al.* (20) for estrogenized ducks, but this difference is probably a consequence of differences in the levels of estrogen used and possibly also of the species difference. More nucleic acid phosphorus

was found in the sera of the pullets receiving both hormones than in the sera of the birds receiving estrogen only, but this difference did not attain significance. It is evident that the testosterone had not any appreciable effect on the level of serum nucleic acid. Thus the reaction of serum nucleic acid to administration of gonadal hormones follows the general pattern of the response of serum calcium and other serum phosphorus fractions, including serum phosphoprotein.

It may be remarked that the orcinol reaction was found to be unreliable when attempts were made to apply Schneider's (28) method to the analysis of the sera. The reasons for this poor agreement between the two methods for nucleic acid in the sera from estrogenized birds have not been fully elucidated, but it is probable that the trichloroacetic acid extracts of the serum samples contained material other than nucleic acid which gave a color with the orcinol reagent.

Experiment II. Changes in the Liver Nucleic Acids of the Domestic Fowl During Normal Growth from Hatching to Sexual Maturity

In view of the results now reported as well as those reported previously (5), it became of interest to investigate possible normal sex differences in the domestic fowl.

A group of cross bred (New Hampshire ♂ × Barred Plymouth Rock ♀) was hatched and reared to sexual maturity. The birds received a commercial chick starter until they were 12 weeks old, when they were given a commercial developer ration. At 15 weeks the birds were placed on good grass range and received a commercial developer ration and scratch grain.

A number of birds were killed at intervals between hatching and attainment of full reproductive activity and the livers removed and analyzed for RNAP and DNAP, total lipid, and crude protein. The total lipid was determined by the procedure described above for total serum lipid. At one day and at six days of age it was necessary to pool the livers from seven chicks in order to secure sufficient material for the desired analytical procedures. From the time the birds were 32 days old, a single liver provided sufficient material.

The average results for this series of determinations are set out in Table VI for male birds and in Table VII for female birds.

It will be noted that the amounts of total lipid in the livers of males and of females were almost the same until the birds approached sexual maturity. The high values for liver lipid during the first 21 days are closely similar to those reported by Bolton (3) in connection with studies of variations in the riboflavin content of liver. From 123 days of age, at which time sexual activity was in evidence in the case of these birds, the liver lipid of females tended to increase while the liver lipid of males did not show any apparent change from the levels previously obtaining. Flock *et al.* (13) and Lorenz *et al.* (19) have also reported that as pullets approach the laying season, so does the liver fat increase. It seems reasonable to ascribe this increase to endogenous

TABLE VI

NUCLEIC ACID CONTENT OF LIVERS OF MALE FOWL FROM HATCHING TO SEXUAL MATURITY (AVERAGE VALUES)

Age in days	No. of observations	Live weight, gm.	Liver weight, gm.	Liver crude protein, % dry matter	Liver total lipid % dry matter	Liver RNAP, mgm. per kgm. live weight	Liver DNAP, mgm. per kgm. live weight	RNAP / DNAP
1	4	38.2	0.86	42.5	42.9	11.9	5.1	2.34
6	4	45.8	1.24	53.3	34.4	19.3	8.2	2.36
13	5	93.7	2.48	65.1	10.4	19.6	8.2	2.39
20	3	139	3.89	69.7	8.9	20.6	8.4	2.48
32	4	288	6.57	69.9	8.6	14.0	7.1	1.73
69	4	987	19.6	67.4	9.8	14.0	5.5	2.52
91	3	1246	25.0	63.5	6.8	16.2	6.7	2.40
123	3	2315	37.9	77.4	10.0	13.8	6.0	2.30
155	0	—	—	—	—	—	—	—
180	5	2483	37.8	78.3	9.0	11.8	6.3	1.86

TABLE VII

NUCLEIC ACID CONTENT OF LIVERS OF FEMALE FOWL FROM MATCHING TO SEXUAL MATURITY (AVERAGE VALUES)

Age in days	No. of observations	Live weight, gm.	Liver weight, gm.	Liver crude protein, % dry matter	Liver total lipid % dry matter	Liver RNAP, mgm. per kgm. live weight	Liver DNAP, mgm. per kgm. live weight	RNAP / DNAP
1	4	36.8	0.87	43.9	43.0	13.2	4.9	2.70
6	4	44.5	1.28	52.4	32.5	21.8	8.6	2.54
13	5	87.1	2.60	67.0	10.4	22.4	9.8	2.24
20	3	135	3.75	66.2	8.0	22.9	9.1	2.52
32	4	304	7.53	66.6	8.5	13.8	7.6	1.82
69	4	936	16.5	72.1	10.4	13.8	5.5	2.48
91	3	1143	19.7	66.3	6.4	13.2	6.0	2.20
123	3	1397	30.0	66.7	8.8	16.3	6.9	2.36
155	6	2109	48.4	57.8	22.5	16.4	6.4	2.51
180	7	2124	45.0	71.9	13.9	17.8	6.6	2.70

estrogen activity, at any rate in part. The liver crude protein of the pullets also increased with the onset of sexual activity. This increase does not appear to have been studied previously.

The ratio RNAP/DNAP was relatively high in the newly hatched chicks, and declined somewhat thereafter. Novikoff and Potter (24) studied the changes in the amounts of RNA and DNA in the whole chick embryo during incubation. They demonstrated relatively high RNA/DNA ratios during the first three days of incubation, the value declining to a minimum at the fifth day, increasing to a maximum around the 15th day and then declining again as hatching approached. Novikoff and Potter related these changes to the

intensity of protein anabolism, as gauged by the protein content of the dry matter of the whole embryo. They reported a RNA/DNA ratio for the whole embryo at hatching of 1.73. The RNA/DNA ratio as calculated for the livers of the newly hatched chicks in Experiment 2 was 2.05. This higher value is presumably due to the fact that the ratio for the liver is higher than that for the embryo as a whole. It seems likely that the changes in the ratio RNAP/DNAP in the livers during incubation follow a similar course to that followed by one embryo as a whole, and that the decline in the ratio in the livers after hatching is a continuation of a decline which proceeds during the last few days of incubation.

While the present observations are admittedly limited, they do confirm the hypothesis that the onset of reproductive activity in the female fowl will affect the liver nucleic acid content of the liver by increasing the RNAP content to a relatively greater degree than the DNAP content, and that this effect will bring about a sex differentiation in liver nucleic acids at this stage. In view of the evidence secured with estrogenized immature pullets, it is not unreasonable to ascribe the major role in determining this sex differentiation to endogenous estrogen production in the sexually active pullet.

Acknowledgments

The financial support for this work provided by the National Research Council of Canada is gratefully acknowledged. Appreciation is also expressed for the award of an Agricultural Institute of Canada Fellowship which supported one author (D.G.C.) during the course of part of the experimental work.

The authors are indebted to the Schering Corporation, Limited, Montreal, and to Charles E. Frosst and Company, Montreal, for generous gifts of hormones.

References

1. ALLAN, J. C. Quantitative study of the effects of estradiol benzoate in modifying the incidence of binucleated cells in the rabbit liver. Endocrinology, 34 : 50-59. 1944.
2. BERENBLUM, I. and CHAIN, E. An improved method for the colorimetric determination of phosphate. Biochem. J. 32 : 295-298. 1938.
3. BOLTON, W. The riboflavin requirement of the White Wyandotte Chick. III. The rates of depletion of the tissues. J. Agr. Sci. 37 : 323-328. 1947.
4. CHAPMAN, D. G., COMMON, R. H., and MAW, W. A. Effects of estrogen and androgen on liver iron of the immature pullet. Sci. Agr. 30 : 194-200. 1950.
5 CHAPMAN, D. G., HANSON, A. A., COMMON, R. H., and MAW, W. A. The effect of gonadal hormones on liver nucleic acids in the immature pullet. Can. J. Research, D, 27 : 200-206. 1949.
6. CHARGAFF, E., VISCHER, E., DONIGER, R., GREEN, C., and MISANI, F. Composition of desoxypentose nucleic acids of thymus and spleen. J. Biol. Chem. 177 : 405-416. 1949.
7. CLAVERT, J. Action de la folliculine sur le foie du pigeon. Compt. rend. soc. biol. 138 : 928-930. 1944.
8. CLAVERT, J., and RANDAVEL, C. Action de la folliculine sur le foie de pigeon: modifications histologiques du noyau de la cellule hepatique et de la basophilie de cytoplasme. Compt. rend. soc. biol. 141 : 1258-1259. 1947.

9. COMMON, R. H., BOLTON, W., and RUTLEDGE, W. A. The influence of gonadal hormones on the composition of the blood and liver of the domestic fowl. J. Endocrinol. 5 : 263-273. 1948.

10. COMMON, R. H., RUTLEDGE, W. A., and HALE, R. W. Observations on the mineral metabolism of pullets. VIII. The influence of gonadal hormones on retention of calcium and phosphorus. J. Agr. Sci. 38 : 64-80. 1948.

11. CORRADETTI, R. The nuclear reaction of Feulgen on platelets. Boll. soc. ital. biol. sper. 8 : 603-605. 1933.

12. ENTENMANN, C., LORENZ, F. W., and CHAIKOFF, I. L. The endocrine control of lipid metabolism in the bird. III. The effects of crystalline sex hormones on the blood lipids of the bird. J. Biol. Chem. 134 : 495-504. 1940.

13. FLOCK, E., BOLLMAN, J., HESTER, H. R., and MANN, F. C. Fatty livers in the goose produced by over feeding. J. Biol. Chem. 121 : 117-129. 1937.

14. GULLAND, J. M., BARKER, G. R., and JORDAN, D. O. The chemistry of the nucleic acids and nucleoproteins. Ann. Rev. Biochem. 14 : 175-206. 1945.

15. HELLER, V. G. and THAYER, R. H. Chemical changes in the blood composition of chickens and turkeys fed synthetic estrogens. Endocrinology, 42 : 161-167. 1948.

16. HERRICK, E. H. Some influences of stilbestrol, estrone and testosterone on the genital tract of young female fowls. Poultry Sci. 23 : 65-66. 1944.

17. KLINE, I. T., DORFMANN, R. I., and MCCREERY, P. Testosterone stimulation of the oviduct in vitamin deficient chicks. Endocrinology, 48 : 39-43. 1951.

18. LEYVA, J. P. Biochemical studies on tuberculosis. II. The nucleic acid reaction of the serum in tuberculosis. J. Philippine Med. Assoc. 24 : 327-333. 1948.

19. LORENZ, F. W., CHAIKOFF, I. L., and ENTENMANN, C. Liver lipides of the laying and non-laying bird. J. Biol. Chem. 123 : 577-585. 1938.

20. MANDEL, P., CLAVERT, J., and BIETH, R. Effets de la folliculine sur les diverses fractions du phosphore protidique du serum chez le canard. Compt. rend. soc. biol. 141 : 1262-1263. 1947.

21. MANDEL, P. and METAIS, P. Les acides nucleiques du plasma sanguin chez l'homme. Compt. rend. soc. biol. 142 : 241-243. 1948.

22. MAXWELL, B. F., MOXON, A. L., and KOHLMEYER, W. The influence of egg production on blood levels of calcium sodium and potassium in turkeys. Proc. S. Dakota Acad. Sci. 26 : 137. 1946-47.

23. MIRSKY, A. E. and RIS, H. Variable and constant components of chromosomes. Nature, 163 : 666-667. 1949.

24. NOVIKOFF, A. B. and POTTER, V. R. Changes in nucleic acid concentration during the development of the chick embryo. J. Biol. Chem. 173 : 233-238. 1948.

25. PARKES, A. S. and EMMENS, C. W. Effect of androgens and estrogens on birds. Vitamins and hormones, 2 : 361-408. 1944.

26. RANNEY, R. E., ENTENMANN, C., and CHAIKOFF, I. L. The lecithin, cephalin and sphingomyelin of plasma and liver of the fowl: their metabolic interrelations as shown by administration of diethylstilbestrol. J. Biol. Chem. 180 : 307-313. 1949.

27. SCHMIDT, G. and THANNHAUSER, S. J. A method for the determination of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissue. J. Biol. Chem. 161 : 85-89. 1945.

28. SCHNEIDER, W. C. Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161 : 293-303. 1945.

29. TABER, E. Androgen secretion in the fowl. Endocrinology, 48 : 6-16. 1951.

30. WOLTERINK, L. F., REINEKE, E. P., and DAVIDSON, J. A. Hemoglobin concentration in the blood of normal and estrogen treated turkeys. Am. J. Vet. Research, 8 : 431-463. 1947.

STUDIES ON THE ENDOPARASITIC FAUNA OF TRINIDAD MAMMALS

VII. PARASITES OF HYSTRICOMORPH RODENTS[1]

By T. W. M. Cameron[2] and M. R. Reesal[3]

Abstract

A possible new variety of cestode, *Raillietina* (*R.*) *demerariensis* var. *trinitatae*, and two new varieties, *Trichuris gracilis* var. *trinitatae* and *Aspidodera binansata* var. *agoutiae* and a new species of nematode, *Helminthoxys urichi* are recorded from the agouti and lappe from Trinidad, B.W.I.

The hystricomorph rodents are, with the exception of the Old World and North American porcupines and the African cave and rock rats, and a few doubtful forms, South American in their distribution and have seen their greatest development in that continent. Only two species, however, occur in the island of Trinidad, B.W.I. These are the "lappe" and the "agouti."

The lappe or paca, *Cuniculus paca* (synonym: *Coelogenys* or *Agouti paca*) is widely distributed through continental South America but does not occur on any of the other West Indian islands. The agouti (*Dasyprocta agouti*), which is also widely distributed on the continent, was, however, present on most islands at the time of the conquest, although it has now been exterminated on most of them; there is at least a strong probability that it was introduced to most of them by pre-Columbian man and that Trinidad, and possibly Tobago, was its only true island home. These animals are closely related to each other and, being of comparatively large size were often—and still are occasionally—used for food. They are fast runners and jumpers and in Spanish times were often hunted like foxes. Both are terrestrial but both are at home in fresh water. This is particularly true of the lappe which at one time was regarded as semiaquatic.

The material described in this paper was collected mainly by the late Professor Urich of Trinidad and the senior author between 1936 and 1939, as described in previous papers in this series. Much of it was recovered from entrails sent to Canada preserved in formalin. Some material was collected by the junior author during the summer of 1949: this was fixed in hot 70% alcohol.

Stichorchis giganteus (Diesing, 1835) Travassos, 1932

This amphistome is not common in Trinidad agoutis. It was recovered from two animals by the senior writer; in one a single specimen was present, in the other, five. It has been redescribed from porcine animals in South

[1] *Manuscript received February 27, 1951.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

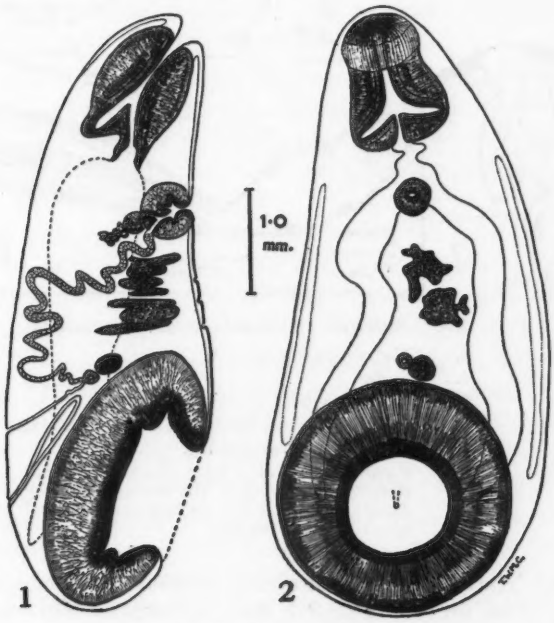
[2] *Director, Institute of Parasitology and Professor of Parasitology, McGill University.*

[3] *Junior Research Assistant.*

America by Vaz (12) and the tamandua (edentate) in Trinidad by Cameron (3), who suggested that this parasite might be normal to the collared peccary which occurs in Trinidad.

The edentate parasite was smaller than those found by Vaz and it is interesting to note that the agouti form, although sexually mature, is even smaller. In length (6 mm.) it agrees with the edentate form, but its maximum width (about 3 mm.) and thickness (about 2 mm.) are less than the corresponding measurements (4.5 and 2.75 mm.) of the edentate parasite. The South American form is much larger being 10 to 12 mm. in length, 4.8 to 6.4 mm. in width, and 3.2 to 3.7 mm. in thickness.

The structure and disposition of the internal organs are the same in all but there is a noticeable reduction in the size of organs corresponding with the reduced body size. Figs. 1 and 2 are ventral and lateral reconstruction of this worm prepared from serial sections.

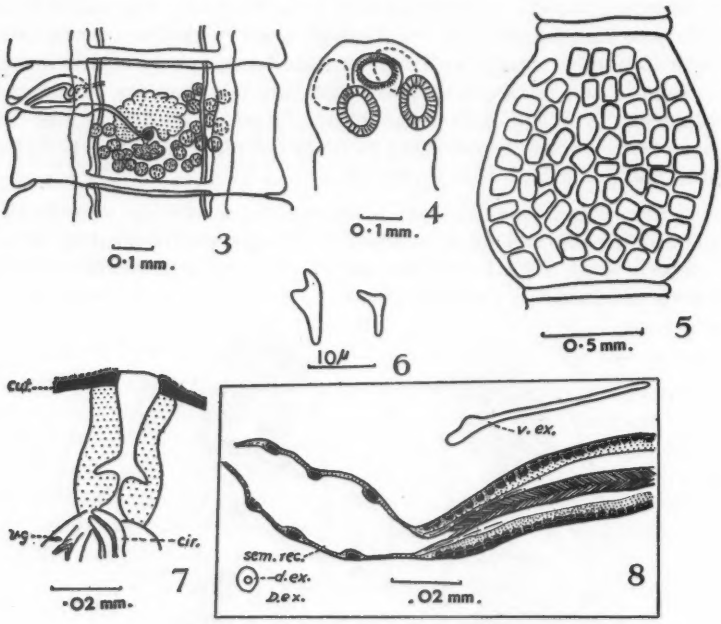


FIGS. 1–2. *Stichorchis giganteus*
1. Ventral viewpoint.
2. Lateral viewpoint.

Raillietina (*R.*) *demerariensis* var. *trinitatae var. nov.*

This tapeworm was recovered from two agoutis and four lappes. The largest specimen (Figs. 3–8) collected measures 60 mm., while the maximum width varies from 1.1 to 1.3 mm.

The scolex measures 0.32 mm. long and is 0.37 mm. in diameter. It is provided with an armed rostellum and four armed suckers. The rostellum has a diameter of 0.13 mm. and is armed with two rows of about 170 hammer-



FIGS. 3–8. *Raillietina* (*R.*) *demerariensis* var. *trinitatae*

3. Mature segment.
4. Scolex.
5. Gravid segment.
6. Left: hook of rostellum; right: hook of sucker.
7. Common genital opening.
8. Proximal end of vagina showing seminal receptacle (sem. rec.). The ventral excretory canal (v. ex.) and the dorsal excretory canal (d. ex.) are also shown.

shaped hooks. The rostellar hooks are quite constant in length being 0.01 mm. long. The suckers are 0.088 to 0.1 mm. in diameter and each is armed with about 12 rows of smaller hooks, about 0.065 mm. long.

The scolex is followed by a comparatively short neck, 0.01 mm. long. The proximal segments are not clearly divided from each other, all being about 0.11 mm. wide and about 0.02 mm. long. As they become more mature the distal portion widens so that the rectangular shape changes to a more trapezoid form. As the uterus develops each segment elongates until it is appreciably longer than it is wide. The gravid segments are about 2 mm. long and 1.2 to 1.4 mm. wide.

The subcuticular musculature of circular and longitudinal muscles appears to be quite normal in distribution and thickness but the vertical musculature is, as Baylis (2) has reported for *R.* (*R.*) *alouattae*, very deficient. The internal longitudinal and transverse muscles are scattered and not arranged into definite fibers. Connecting dorsoventral fibers run the thickness of the proglottis. The dorsal excretory canal is very narrow (0.006 mm.) in contrast with the ventral excretory vessel which is very wide (0.6 mm.). The former is more medial in position.

The genital pores are unilateral, opening at the junction of the middle and proximal third of the segment. There is a small common genital duct—0.3 to 0.4 mm. in length. The male and female ducts pass between the dorsal and ventral excretory vessels. A conspicuous cirrus sac is present. It is pyriform and curved with a length of 0.14 to 0.2 mm. and maximum width of 0.07 mm. containing a narrow and unarmed cirrus (0.10 mm.). In its proximal region the cirrus sac contains a small portion of the vas deferens. The lumen of the vagina is provided with outwardly pointing bristles, the vagina itself having a diameter of 0.02 mm.

There are from 28 to 32 testes with an average diameter of 0.035 mm. About 18 to 20 are always present in the antiporal field, four or five posterior to the vitelline gland and seven to eight posterior to the genital ducts. Testes are never found anterior to the vas deferens though they may be dorsal to the ventral excretory canal.

The ovary is rosette-shaped and is situated at the center of the segment. It has a width of 0.2 mm. when fully developed. Posterior to the ovary is the vitelline gland, 0.09 mm. wide; between the ovary and vitelline gland and lying somewhat dorsally is a small shell gland.

The gravid segments are barrel-shaped and are packed with capsules containing eggs. There are about 50 to 70 capsules per segment, each having a diameter of about 0.1 mm. Details of the capsules were difficult to make out as the gravid segments did not stain well. However, there appeared to be about 8 to 12 eggs in each capsule, each egg having a diameter of 0.01 mm.

This tapeworm is closely related to *R.* (*R.*) *demerariensis* (Daniels, 1895) first described from man in British Guiana and subsequently from howler monkeys (7) in adjacent Dutch Guiana. However, it is also closely related to *R.* (*R.*) *alouattae* Baylis, 1947, found in howler monkeys in Dutch Guiana. The differences between these forms and those of the writers are mainly numerical and are summarized in Table I.

There seem to be no characteristics except size separating these four forms and there appears to be considerable variation in dimensions and numbers of hooks, testes, and egg capsules even within the tapeworm from the same host. It seems probable that all represent a single species, but pending further study of the problem and to prevent subsequent confusion, the writers' material is regarded as a new variety (var. *trinitatae*) of *R.* (*R. demerariensis*).

TABLE I

	R. demerariensis Man, B.G.	*R. demerariensis Alouatta senecula*, D.G.	*R. alouattae A. macconnelli*, D.G.	Present form agouti and paca Trinidad
Scolex	200μ	560–600μ	450–620μ	320 × 370μ
Suckers	92–100/50–70μ	166μ	120–190μ	88–100μ
Rostellum	85μ	234μ	100–150μ	132μ
No. of hooks	150–200	180–200	170–224	170
Length of hooks	14–20μ	18–23μ	15–17μ	11μ
No. of testes	140–86	65–75	110–130	28–32
Cirrus pouch	180–360μ × 72–90μ	252–288μ × 108μ	220–250μ × 110μ	200–140μ × 70μ
No. of egg capsules	70–350	180	38–160	50–70
Total length	1000 mm.?	250–300 mm.	130–340 mm.	60 mm.
Maximum width	3 mm.	8 mm.	7 mm.	1–3 mm.

There seems less doubt, however, that all these forms, if not identical, have at least a common origin.

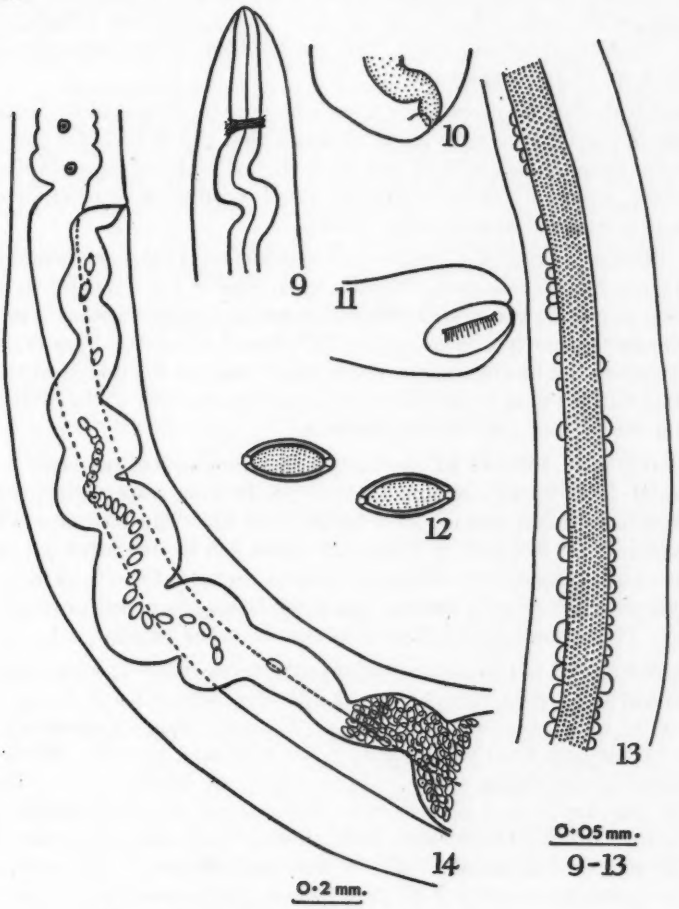
It is interesting to note that three species of this subgenus of tapeworms have been reported from man in Ethiopian and oriental regions and of these, two have also been found in rodents. Only the South American species occurs in primates other than man, and South American monkeys are known to be hosts to numerous aberrant parasites—forms which occur normally in marsupials and edentates—and they are the only monkeys known to harbor human lice. Our recovery of this tapeworm suggests strongly that in this case both monkeys and man are accidental, or at least, recent hosts and that, as is the case in the Old World, rodents are the natural hosts. While both murid and sciurid rodents occur in Trinidad and continental South America and we are largely ignorant of the nature of their endoparasites, the fact that two agouti and four lappes were found infected suggests that this parasite is indigenous to the hystricomorph rodents of South America and was not introduced into that continent in human beings.

Strongyloides agoutii Griffiths, 1940

Strongyloides agoutii is very common among Trinidad agoutis; four out of six animals examined carried the parasite. It has been described adequately by Griffiths (5) who was able to induce infections in the guinea pig. However, it should be noted that while the coiling of the ovaries is variable among parasitic females with an accelerated life cycle in the guinea pig, it is not variable when the parasite is in its normal host—the agouti. In this animal there are two conspicuous coils around the intestine posterior to the vulva, and none anterior to the vulva. This is seen sometimes in the guinea pig, but in many cases egg production has started so early that the coiling of the ovary is interrupted.

Trichuris gracilis var. *trinitatae var. nov.* (Figs. 9–14)

Whipworms were found in six agouti from Trinidad but only females were present.



FIGS. 9–14. *Trichuris gracilis* var. *trinitatae*

9. Ventral–lateral view of head end.
10. Posterior extremity.
11. Ventral view of vulva.
12. Eggs.
13. Ventral view of bacillary band at the level where the cuticular plaques begin to appear.
14. Ovejector.

Rudolphi (10) found a whipworm in the agouti from Brazil and named it *Trichocephalus gracilis*. Only female specimens were present and his description is essentially as follows: whitish, capillary portion darkening anteriorly;

head acute; anterior oesophageal portion of body scarcely longer than posterior portion; length—47 to 54 mm. Posterior portion of body relatively slender, slightly curved, blunt posteriorly. The worm was later placed in the genus *Trichuris* by Hall (6). Baylis (1) in a list of worms from Dutch Guiana records, "*Trichuris? gracilis* (Rud. 1819) on the basis of two female specimens recorded from *Dasyprocta agouti*".

Although Rudolphi's description is not adequate for proper identification on the basis of female specimens alone, it seems that the Trinidad worm differs sufficiently from that existing in agouti on the mainland of South America to keep them separate. Accordingly, to avoid possible confusion in future studies, it is regarded as a separate variety.

The posterior portion of *T. gracilis* var. *trinitatae* is creamy-white and sickle-shaped when freshly collected, although when fixed it has a much less regular form than *T. gracilis* var. *gracilis* which is longer and more uniform in its bend. Not infrequently our specimens appear "S"-shaped or wavy. Very fine transverse striations can be discerned in the posterior part of the body but they are much less distinct than in the filamentous anterior portion of the body where they give the outline a serrated appearance.

The worm is 38.56 to 39.62 mm. long. The anterior and posterior portions measure 21.52 to 27.44 mm. and 15.10 to 18.40 mm., respectively, the ratio of the one to the other varying from 13/10 to 16/10. The maximum width of the posterior part is 0.6 to 0.7 mm.; in the region of the vulva it is 0.3 to 0.4 mm., and at the middle of the oesophageal region 0.10 to 0.14 mm. The head end is usually twisted dorsally and small lateral shoulders can sometimes be seen. The nerve ring, is 0.06 to 0.07 mm. from the anterior end.

A bacillary band begins just posterior to the nerve ring. It widens to about one-third of the body circumference (0.021 to 0.32 mm.) and disappears at the junction of oesophagus and intestine. Cuticular plaques appear on either side of the bacillary band commencing 0.3 to 0.45 mm. from the anterior end, and extending for almost 4 mm. At first they are irregular in position but later on they are packed against each other. They are also irregular in size varying from 0.006 to 0.015 mm. in diameter. Beginning at about 1.2 mm. from the anterior end, small studlike structures, 0.03 mm. in diameter, can be seen at regular distances of 0.22 to 0.23 mm. from each other. The nature and function of these structures are not known.

The vulva is slightly posterior (0.70 mm.) to the level of the oesophagus–intestinal junction. It is usually flush with the side of the body but in some cases the lips are slightly evaginated. In face view, it is quite simple. The ovejector is long and muscular with an average length of 2.79 mm. and width of 0.17 mm. The wide uterus is connected to the ovejector by a narrow cylindrical channel. The distance of the posterior loop of the uterus from the anal end varies from 0.9 to 1.53 mm.

The eggs are 0.050 to 0.059 by 0.23 to 0.28 mm. and are of fairly constant dimensions.

The anus is subterminal in position and ends between two small lateral lobes at the end of the body.

This worm differs consistently in body size from the mainland variety. The posterior part of the body in *T. gracilis trinitatae* is always well under 20 mm. whereas it is always appreciably higher in *T. gracilis gracilis* (22 to 26 mm.). Maximal total lengths are 45 mm. and about 60 mm., respectively.

While these differences are constant they are not sufficiently significant to warrant creation of a new species. They may have arisen from a relatively recent geographical isolation and placing them into separate species would undoubtedly obscure the close relationship between the two forms.

A few broken females of whipworms were also found in the lappe but it is difficult to say whether they are the same as those in the agouti.

It is of interest to note that no male worms have been found in either host by any investigator.

Acanthocheilonema sp.

A single specimen was found in the body cavity of one agouti.

The worm is a female 51.2 mm. in length and 0.20 mm. in width. It has a wide mouth with two lips (dorsoventral). The sides of the lips project outward giving the appearance of small shoulders. At the posterior extremity there are two prominent cone-shaped structures which give the tail a bifid appearance.

Specific diagnosis is not possible without males although the generic characters are quite evident.

Acanthocheilonema perstans occurs in man in Trinidad although it is a much longer parasite—70 to 80 mm. Monkeys, which are usually the natural reservoirs of these parasites, are represented in Trinidad by two species—the red howler (*Alouatta insularis*) and a capucin (*Cebus appela*). No filaria worms were seen in the single howler monkey examined.

A female specimen of this genus was recovered from a tamandua by Cameron (3) and it may be the same species as recovered from the agouti.

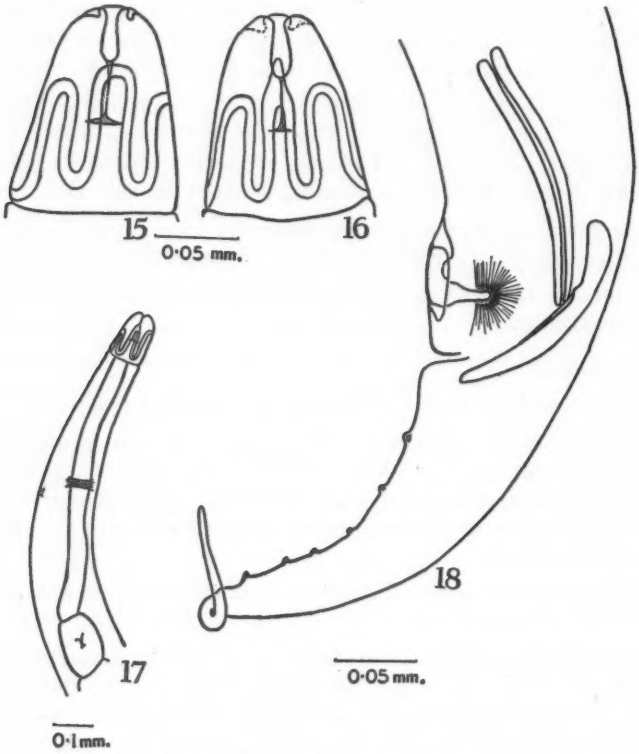
Trichostrongylidae

Every agouti autopsied yielded trichostrongyles, the majority belonging to one of two species: *Fuellebornema agoutii* and *Pudica pudica*. These parasites have been described adequately by Travassos (11) and the present authors have nothing further to add to his description. The lappe, on the other hand, harbored a single species of trichostrongyle—*Heligmostrongylus sedecimradiatus*—a species which sometimes occurs in the agouti also.

Aspidodera binansata var. *agoutiae var. nov.* (Figs. 15–18)

Three males in a good state of preservation were recovered from the intestine of an agouti. In total length the worms vary from 3.37 to 3.42 mm. and from

0.22 to 0.25 mm. in maximum width. The body is elongated "S"-shaped with the anterior quarter bent dorsally and the posterior quarter ventrally. The cuticle is transversely striated.



FIGS. 15–18. *Aspidodera binansata* var. *agoutiae*
15. Dorsal view of head end.
16. Ventral view of head end.
17. Lateral view.
18. Lateral view.

Lateral alae extend from the posterior end of the cephalic collar to about 0.1 mm. from the anus.

The mouth leads into a vestibule, 0.74 mm. in length. There are three conspicuous lips; a dorsal and two subventral. The junction of the vestibule and oesophagus is about 0.06 mm. from the anterior end. Measured from this junction and including the posterior bulb, the oesophagus is 0.8 to 0.9 mm. in length. The posterior bulb is 0.15 mm. long and 0.14 mm. wide.

The head end is ornamented with festoons, the nature and significance of which are not clear. There is fluid within the festoons and this had been observed to move when pressure was applied. The arrangement around the

head is similar to that in *A. binansata* Railliet and Henry, 1913 (9) from South American edentates. However, on the dorsal side, the posterior loops do not extend to the rim of the cephalic collar and the dorsal loop extends further anteriorly than the two laterodorsal loops (Figs. 15–16). The cephalic collar on which the festoons lie is 0.12 mm. long.

The excretory pore is 0.4 to 0.5 mm. from the anterior end, opening on a conspicuous knob. The nerve ring is at the level of the excretory pore.

The ventral sucker, with a strong chitinous ring, except in the posterior margin where it is weaker, has a peculiar gutter which is pictured in Proenca's drawing (8) of *A. binansata.* The muscles seem to radiate rather from this area than from the periphery of the sucker. The diameter of the sucker is 0.042 to 0.044 mm., its depth is 0.012 to 0.015 mm. and the depth of the gutter is 0.021 to 0.028. The distance from the anus is 0.045 mm. The tail is slender and relatively long, measuring 0.34 to 0.37 mm. in length; its width at the proximal end is 0.09 to 0.11 mm. Smaller, inconstant papillae are present in addition to larger papillae, one pair of which is found just anterior to the sucker, one pair immediately preanal, and seven pairs postanal.

There are two equal spicules, not strongly chitinized, measuring 0.16 to 0.17 mm. They are 0.15 mm. at their proximal end, slightly wider at the center and end abruptly in a blunt point. This parasite has, therefore, more slender and shorter spicules than *A. binansata binansata* from edentates (0.280 to 0.304 by 0.029 to 0.32 mm.). However, the gubernaculum of the rodent form is longer than that in the edentate, being 0.140 to 0.147 mm. compared with 0.096 mm. In width it is more slender—0.012 mm. compared with 0.014 mm.

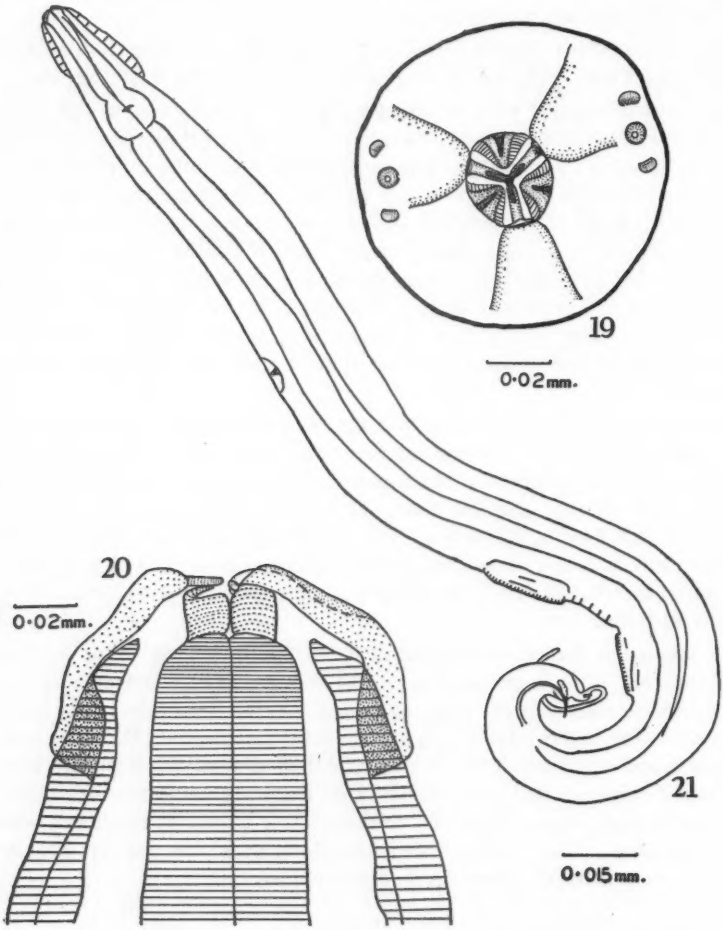
All species of the genus *Aspidodera* from South America have been found in edentates. This is the first record of this group in a rodent. It is quite possible that these worms represent an accidental infection of *A. binansata* from edentates in Trinidad which are known to be infected (3). However, the worms described above differ in many of their dimensions from the edentate worm and there is nothing to indicate that these are immature specimens. It is thought inadvisable, therefore, to make these worms synonymous with the edentate type. They are recorded, therefore, as a new variety, *agoutiae*, in distinction to those of edentates *A. binansata* var. *binansata.*

Helminthoxys urichi sp. nov. (Figs. 19–24)

A large number of males and females was recovered from the large intestine of an agouti. The male (Fig. 21) is 3.0 to 4.3 mm. in length and 0.22 to 0.32 mm. in width. The female is larger: 4.8 to 9.7 mm. long.

Conspicuous transverse striations about 0.04 to 0.06 mm. apart occur on the entire body length in both sexes, becoming closer in the posterior region. Both sexes have cephalic alae but they are more conspicuous in the female. The alae begin posterior to the vestibule and usually attain a length of 0.15 mm. but may reach the level of the oesophageal bulb.

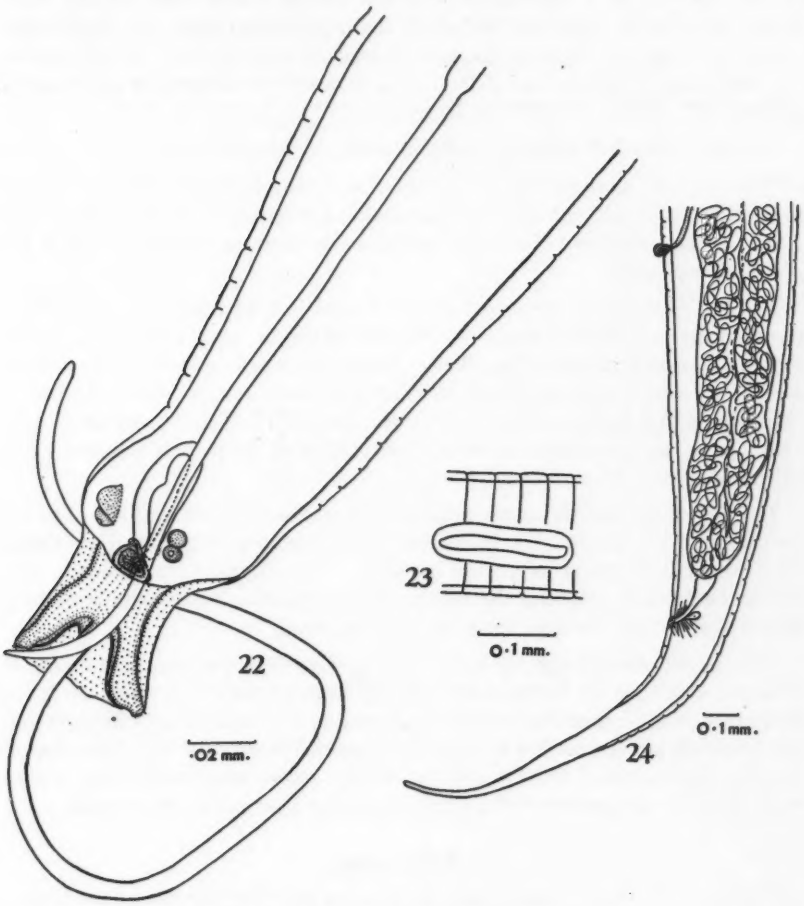
The male bears two very conspicuous mamelons posteriorly. The more anterior, measuring 0.15 to 0.18 mm. in length, is 0.97 to 1.27 mm. from the cloaca. It is usually larger than the more posterior one which is 0.13 to 0.16



FIGS. 19–21. *Helminthoxys urichi*
19. "En face" view of head end.
20. Lateral view of head end.
21. Lateral view of male.

mm. in length and 0.65 to 0.89 mm. from the cloaca. Distances from the cloaca are measured from the middle of the mamelon. The structure of the ventral surface of a mamelon is shown in Fig. 23.

There is a very definite vestibule (0.18 mm. deep) surrounded by three fleshy lobes. These are dorsal and subventral in position. Directly over each lobe is a liplike structure which, when compared with the lips in other



FIGS. 22–24. *Helminthoxys urichi*

22. Ventral view of tail (male).
23. Ventral view of mamelon (male).
24. Lateral view of posterior end of gravid female. Note persistence of plug on vulva.

genera and related to the position of the amphids, can be designated as true lips. Alternating with these are filamentous projections, the supralabia, which seem to be mainly cuticular in structure. Since the supralabia are clearly

anterior to the true lips in lateral view, the latter may be mistaken for interlabia. The lip structure in this species closely resembles that in *Wellcomia evoluta* from the Canadian porcupine.

The oesophagus is claviform with an almost round oesophageal bulb. Measured from the head and including the oesophageal bulb, the oesophagus is 0.35 to 0.44 mm. long in the male and 0.44 to 0.53 mm. in the female. The oesophageal bulb is 0.11 to 0.15 mm. in diameter in the male and slightly larger in the female—0.13 to 0.16 mm.

The nerve ring is 0.120 to 0.137 mm. from the anterior end.

The excretory pore in the male is about 0.9 mm. from the anterior end; in the female, however, the distance is variable measuring between 0.5 and 0.8 mm. Around the pore there is a semicircular clearance through which the pore is conspicuous.

The tail of the male is curved ventrally and is 0.41 mm. long including a terminal filament of 0.35 mm. At the root of the terminal filament on either side are conspicuous lateral papillae. There is a single spicule (0.23 to 0.25 mm. long) and a gubernaculum (0.45 to 0.47 mm. long). Both are poorly chitinized and it is often difficult to see the proximal end of the spicule clearly. Distal to the gubernaculum on the ventral surface of the body is a fishhook-like chitinous piece with prominent prongs.

The tail of the female tapers gradually to a slender point and is 1.2 to 1.4 mm. in length. The vagina is slender and vestigial having a small chitinous plug which is retained in gravid females. The vagina runs forward, then curves backwards reaching the vulva in the posterior half of the body at a distance of 1.1 to 1.6 mm. from the anal opening.

This is the second species in the genus *Helminthoxys* the first being *H. caudatus* described by Freitas and Lent (4) from *Caviella australis*. *H. urichi* is distinct from *H. caudatus* in its measurements, and in its labial configuration, the vestibule being guarded by a distinct finger-like supralabia. Both species can be distinguished from *Syphacia*, whose males also carry two ventral mamelons, by the presence of a small vestibular opening at the mouth.

References

1. BAYLIS, H. A. Some roundworms and flatworms from the West Indies and Surinam. I. Nematodes and Acanthocephala. J. Linnean Soc. London, Zool. 41 : 394-405. 1947.
2. BAYLIS, H. A. Some roundworms and flatworms from the West Indies and Surinam. II. Cestodes. J. Linnean Soc. London, Zool. 41 : 406-414. 1947.
3. CAMERON, T. W. M. Studies on the endoparasitic fauna of Trinidad mammals. VI. Parasites of Edentates. Can. J. Research, D, 17 : 249-264. 1940.
4. FREITAS, J. F. T. de, LENT, H., and ALMEIDA, J. L. de. Peguena contribuicão ao estudo da fauna helminthologica da Argentina (Nematoda). Mem. Inst. Oswaldo Cruz, 32 : 195-210. 1937.
5. GRIFFITHS, H. J. Studies on *Strongyloides agoutii* from the agouti (*Dasyprocta agouti*). Can. J. Research, D, 18 : 173-190. 1940.
6. HALL, M. C. Nematode parasites of mammals of the orders Rodentia, Lagomorpha and Hydracoidea. Proc. U.S. Natl. Museum, 50 : 1-258. 1916.

7. JOYEUX, Ch. and Baer, J. G. (L'hôte normal de *Raillietina* (*R*) *demarariensis* (Daniels, 1895) en Guyane Hollandaise. Acta Tropica, Basle, 6 : 141-144. 1949.
8. PROENCA, M. C. Revisão do genero *Aspidodera* Railliet and Henry, 1912. Mem. Inst. Oswaldo Cruz, 32 : 427-438. 1937.
9. RAILLIET, A. and HENRY, A. Observations sur les nematodes *Aspidodera* R. and H., 1912. Bull. Museum Natl. Hist. Nat. (Paris), 2 : 93-99. 1913.
10. RUDOLPHI, C. A. Entozoorum synopsis cui accedunt mantissa duplex et indices locupletissimi. Berolina. 1819.
11. TRAVASSOS, L. Revisão da familia Trichostrongylidae Leiper, 1912. Monographs Inst. Oswaldo Cruz, No. 1. 1937.
12. VAZ, Z. Adaptação ao porco domestico *Stichorchis giganteus*, parasita de porros selvagens. Rediscripção. Arch, Inst. Biol. 6 : 45-52. 1935.

PARASITES OF FRESHWATER FISH

V. PARASITIC HELMINTHS OF THE MUSKALLUNGE, *ESOX M. MASQUINONGY* MITCHILL, IN THE ST. LAWRENCE WATERSHED[1]

By L. P. E. Choquette[2]

Abstract

The incidence of the following species of helminths recovered from the digestive tract of 218 muskallunge, *Esox m. masquinongy*, from various localities in the St. Lawrence watershed is recorded: *Azygia augusticauda, A. longa, Triaenophorus nodulosus, Proteocephalus pinguis, Neoechinorhynchus cylindratus, Leptorhynchoides thecatus, Metabronema salvelini*, and *Rhaphidascaris canadensis*. One hundred and ninety-two, or 88% of the fish examined were found to harbor one or more species. The most commonly found species were *T. nodulosus* and *A. longa*. In all cases the number of worms recovered per host was small.

Introduction

Esox m. masquinongy Mitchill is a common game fish in the St. Lawrence River and its tributaries and in recent years an effort has been made by the Office of Biology of the Quebec Department of Fish and Game to increase its population. With this programme in mind a logical step was to secure data pertaining to the breeding and feeding habits, growth, and diseases of this fish.

Materials and Methods

The digestive tracts of fish secured from anglers by the Office of Biology of the Province of Quebec during the muskallunge season (July–September) of 1949 were preserved in 10% formol and sent to the Royal Ontario Museum of Zoology, where the stomach contents were examined, but worms, if any, were left *in situ*. The intestines were left intact. Both stomachs and intestines were then sent to the Institute of Parasitology where the worms were removed and prepared for examination. A total of 218 fish obtained from various localities was thus examined. Most (164) of these were taken from the Lake of Two Mountains (41), Lake St. Francis (77), and Lake St. Louis (46).

Comparatively little is known concerning the helminths in *Esox m. masquinongy*. This is undoubtedly due to the restricted distribution of this host and to the difficulty in securing it. Apart from sporadic reports of parasitic infections the only data on incidence are those of Fischthal (8), Bangham (1), and Mueller (20). The first of these authors examined only four fishes from two lakes in Wisconsin, the second examined 17 fishes from three lakes and hatching points also in Wisconsin, but only three of the fish were mature

[1] *Manuscript received April 20, 1951.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, P.Q., with financial assistance from the National Research Council of Canada.

[2] *Research Assistant and Lecturer.*

individuals from lakes, the remainder being immature fish from hatching ponds. Mueller gives no indication as to the number of fish he examined from Chautauqua Lake, New York. The following species of helminths have been recorded from Canada (Ontario and Quebec) and the United States.

	Number of times recorded in this paper:
Trematoda	
Gyrodactyloidea (8)	—
Neascus sp. (8)	—
Azygia augusticauda (Stafford, 1904) (1)	3
Azygia longa (Leidy, 1851) (24, 3)	117
Cryptogonimus chyli (1)	—
Phyllodistomum staffordi (13)	—
Cestoda	
Proteocephalus ambloplites (8)	—
Proteocephalus pinguis LaRue, 1911 (8, 1, 20)	22
Triaenophorus nodulosus (Pallas, 1781) (8, 1, 4)	172
Acanthocephala	
Neoechinorhynchus tenellus (8, 1)	—
Neoechinorhynchus cylindratus (Van Cleave, 1913) (20)	25
Leptorhynchoides thecatus (Linton, 1891) (1, 20, 12)	14
Nematoda	
Camallanus oxycephalus (8)	—
Philometra sp. (1)	—
Rhaphidascaris canadensis Smedley, 1933 (23)	17
Metabronema salvelini (Fujita, 1920) (2)	1

Discussion

One hundred and ninety-two, or 88%, of the 218 fish examined, were found to harbor one or more species of parasitic helminth. A total of six species of helminths, one of which is undoubtedly an accidental parasite in this host, has been found. Trematodes were found in 120 cases, cestodes in 172, acanthocephalans in 39, and nematodes in 18.

Trematodes

One hundred and twenty of the 192 infected fish, or 62.4%, were found to harbor species of trematodes, but in only 13 cases were they the sole parasites found. Only two species were present, both belonging to the genus *Azygia*

Looss, 1899, namely *A. longa* (Leidy, 1851) and *A. augusticauda* (Stafford, 1904). *A. longa* was the commoner although in all infected fish the number of worms was very small: *A. augusticauda* always occurred in fish also infected with *A. longa*.

Van Cleave and Mueller (26) and Hunter and Rankin (10) have shown that species of *Azygia* occur in a variety of hosts, including species of Esocidae. The earliest records of species of *Azygia* in *Esox m. masquinongy* are those of Stafford (24) and Cooper (3) who described, under various names, species which Manter (14) and Miller (16) have shown to be *A. longa*. Recently, Bangham (1) reported *A. augusticauda* from this host from Wisconsin, but no species belonging to this genus were found by Fischthal (8), who also examined hosts from that state, nor by Mueller (20) in his study of the parasites of the muskallunge in New York State.

In their study of *A. longa* and *A. augusticauda* in fish in Oneida Lake, New York State, Van Cleave and Mueller (26) conclude that there is a relationship between these species as encountered in the host and its habitat, *A. augusticauda* being found in shallow- or shore-water fish, and *A. longa* in deep-water fish. This does not appear to be the case in the present study, where *A. longa* is commonly encountered in a shallow-water host, although in no case were the worms numerous. Lyster (13) found *A. longa* in the pike (*Esox lucius*)—a shallow-water species—and in the eel (*Anguilla rostrata*), a deep-water fish, in the St. Lawrence, although in the last host the specimens were more numerous, while Miller (15) found *A. augusticauda* in the small mouth black bass (*Micropterus dolomieu*) and in the doré (*Stizostedion vitreum*).

Cestodes

Two species of cestodes, *Triaenophorus nodulosus* and *Proteocephalus pinguis*, were found in 172 of the fish, both adult and immature stages being present. Thirty-nine fish were found to harbor only cestodes and in only 22 cases were both species of cestode found in the same host; *T. nodulosus* was the commoner one and usually in an immature stage.

Triaenophorus nodulosus is a common cestode in many species of fish both in the United States and Canada. The adult form is found in species of piscivorous fish and its larval stage in a variety of fish upon which the former feed. The first record of this species in *Esox m. masquinongy* is that of Cooper (4) who reported it from Ontario; it has also been recorded in the same host by Bangham (1), Fischthal (8), and Mueller (20).

The life history of this cestode in western Canada has been particularly studied by Miller (17, 18, 19) but no data are available on its biology in eastern Canada. In western Canada also, the life history of other species of this genus has been studied by Newton (21) and by Ekbaum (7). These authors have shown seasonal variations in the development of these cestodes and their incidence in known definitive hosts. This seasonal variation appears to be

suggested in the present study as the forms encountered were predominantly immature. Similar findings are reported by Michajlow (in 17) in his study on the incidence of *T. nodulosus* in pike in Poland.

Proteocephalus pinguis was first reported from Esocidae by LaRue in 1911 (11). It has since been reported in its adult or larval stages by several authors from other hosts and from the muskallunge by Bangham (1) and Fischthal (8) in Wisconsin; Mueller (20) found it to be a common parasite in the larger specimens of muskallunge of the Chautauqua Lake, New York. In the present study it was found only in 22 fish and in every case the number of worms recovered was quite small. Hunter (9), who has made a particular study of the life history of this parasite, believes that two modes of infection are possible. The first intermediary hosts are species of Entomostraca and young fish become infected by eating the crustacea, the procercoids becoming mature in the intestine. If fish are older, however, the procercoid remains in the intestine without further development and such fish may act as passive carriers. The second mode of infection would, therefore, be found in large fish which feed on these carriers, with intestinal procercoids obtained directly from crustacea.

According to Mueller (20) this infection may be an important factor in the rearing of young muskallunge. As the fish in this study were all adults and thus piscivorous in their habits, it is probable that the mode of infection is the second one put forward by Hunter (10). Data (22) on the feeding habits of the muskallunge based upon the examination of full stomachs of 21 adult fish showed that fish constitutes 91% of the total weight of the food found in muskallunge stomachs. The following species of fish from the stomachs were identified: *Ameiurus nebulosus*, *Pomolobus pseudoharengus*, *Perca flavescens*, *Moxostoma* sp., and *Esox* sp.

Acanthocephala

Two species of thornyheaded worms were recovered but in no case were both species found in the same host. Two fish were found to harbor only such worms. *Neoechinorhynchus cylindratus* was the species more commonly encountered. This was also found by Mueller (20) in *Esox m. masquinongy* from Chautauqua Lake, although Bangham (1) and Fischthal (8) in Wisconsin reported a different species of the same genus, namely *N. tenellus*. The other thornyheaded worm recorded, *Leptorhynchoides thecatus*, has been found by these authors in this host both from Wisconsin and New York, as well as by Lincicome and Van Cleave (12) from an unmentioned locality in Ontario.

While the biology of these two species of acanthocephalans has been studied by Ward (27) and DeGuisti (5, 6), lack of local data renders it difficult to explain this relatively low incidence of infection in the St. Lawrence area.

Nematodes

No representatives of this group were found by Mueller (20) in *Esox m. masquinongy* in New York, although Bangham (1) and Fischthal (8) recorded species of nematodes from this host in Wisconsin. Of the two species recorded

in the present study there is no doubt that *M. salvelini* (Fujita, 1920) is not a natural parasite of the muskallunge. It is found commonly in the speckled trout in the eastern part of North America and it has been shown by the writer (2) to be widely distributed throughout the world in species of Salmonidae; no species of *Metabronema* have ever been previously recorded from Esocidae. It was found only once, a single male and an immature female being present, and in this particular case it is possible that the muskallunge had ingested a trout which had gone astray from its normal habitat.

The other nematode found, *Rhaphidascaris canadensis*, was also relatively uncommon and found only in 17 fish, always in small numbers, and in conjunction with other species of helminths. This nematode has been reported by Smedley (23) who found it to be quite common in *Esox lucius* in western Canada but never very abundant. Thomas (25) has shown that part of the development of this nematode takes place in minnows and perch which, in turn, are eaten by the definitive host.

References

1. BANGHAM, R. V. Parasites of northern Wisconsin fish. Trans. Wisconsin Acad. Sci. 36 : 291–325. 1944.
2. CHOQUETTE, L. P. E. On the species of the genus *Metabronema* Yorke and Maplestone, 1926, parasitic in trout and char. Can. J. Research, D, 26 : 329-333. 1948.
3. COOPER, A. R. Trematodes from marine and freshwater fishes, including one species of ectoparasitic turbellarian. Trans. Roy. Soc. Can., V, 9 : 181-205. 1915.
4. COOPER, A. R. North American pseudophyllidean cestodes from fishes. Illinois Biol. Monograph, 4 : 288-541. 1918.
5. DEGUISTI, D. L. Preliminary note on the life cycle of *Leptorhynchoides thecatus*, an acanthocephalan parasite of fish. J. Parasitol. 25 : 180. 1939.
6. DEGUISTI, D. L. The life cycle of *Leptorhynchoides thecatus* (Linton) an acanthocephalan of fish. J. Parasitol. 35 : 437-460. 1949.
7. EKBAUM, E. K. On the maturation and the hatching of the eggs of the cestode *Triaenophorus crassus* Forel from Canadian fish. J. Parasitol. 23 : 293-295. 1937.
8. FISCHTHAL, J. H. Parasites of northwest Wisconsin fishes. Trans. Wisconsin Acad. Sci. 37 : 157-220. 1945.
9. HUNTER, G. W., III. Life history studies on *Proteocephalus pinguis* La Rue. Parasitology, 21 : 487-496. 1929.
10. HUNTER, G. W., III. and RANKIN, J. S. Jr. Parasites of northern pike and pickerel. Trans. Am. Fisheries Soc. 69 : 268-272. 1939.
11. LARUE, G. R. A revision of the cestode family Proteocephalidae. Illinois Biol. Monograph, 1 : 1-351. 1914.
12. LINCICOME, D. R. and VAN CLEAVE, H. J. Distribution of *Leptorhynchoides thecatus* a common acanthocephalan parasitic in fishes. Am. Midland Naturalist, 41 : 421-431. 1949.
13. LYSTER, L. L. Parasites of freshwater fish. I. Internal trematodes of commercial fish in the central St. Lawrence watershed. Can. J. Research, D, 17 : 154-168. 1939.
14. MANTER, H. W. Some North American fish trematodes. Illinois Biol. Monograph, 10 : 127-264. 1926.
15. MILLER, M. J. Parasites of freshwater fish. III. Further studies on the internal trematodes of fish in the central St. Lawrence watershed. Can. J. Research, D, 18 : 423-434. 1940.
16. MILLER, M. J. A critical study of Stafford's report on "Trematodes of Canadian fishes" based on his trematode collection. Can. J. Research, D, 19 : 28-52. 1941.
17. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. I. Introduction and the life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the definitive host, *Esox lucius*. Can J. Research, D, 21 : 160-170. 1943.

18. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake, Alberta. II. The eggs, coracidia, and life in the first intermediate host of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas). Can. J. Research, D, 21 : 284-291. 1943.

19. MILLER, R. B. Studies on cestodes of the genus *Triaenophorus* from fish of Lesser Slave Lake Alberta. III. Notes on *Triaenophorus nodulosus* (Pallas) in the second intermediate host. Can. J. Research, D, 23 : 1-5. 1945.

20. MUELLER, J. F. Parasitism in fishes in the Allegheny and Chemung areas. Supplement to 27th Annual Report New York State Conservation Department, 214-225. 1938.

21. NEWTON, M. V. B. The biology of *Triaenophorus tricuspidatus* (Bloch, 1779), in western Canada. Contribs. Can. Biol. and Fisheries, 7 (n.s.) : 341-360. 1932.

22. Office de Biologie, Ministère de la Chasse et Pêche, Province de Québec. Apercu général de la nourriture du maskinongé (*Esox m. masquinongy*) d'après spécimens récoltes au cours de l'été. (Rapport préléminaire.) Unpublished data. 1950.

23. SMEDLEY, E. M. Nematode parasites from Canadian marine and fresh-water fishes. Contribs. Can. Biol. and Fisheries, 8 : 170-179. 1933.

24. STAFFORD, J. Trematodes from Canadian fishes. Zool. Anz. 27 : 481-495. 1904.

25. THOMAS, L. J. Life cycle of *Rhaphidascaris canadensis* Smedley, 1933, a nematode from the pike, *Esox lucius*. J. Parasitol. 23 : 572. 1937.

26. VAN CLEAVE, H. J. and MUELLER, J. F. Parasites of Oneida Lake fishes. Part 3. A biological and ecological survey of the worm parasites. Roosevelt Wild Life Ann. 3 : 1-334. 1934.

27. WARD, H. L. Studies on the life history of *Neoechinorhynchus cylindratus* (Van Cleave, 1913) (Acanthocephala). Trans. Am. Microscop. Soc. 59 : 327-347. 1940.

CANADIAN JOURNAL OF ZOOLOGY

Notes to Contributors

GENERAL: Manuscripts should be typewritten, double spaced, and the **original and one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. If authors require changes to be made after the type is set, they will be charged for changes that are considered to be excessive. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT: **An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:* All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil **(do not use typewriting)** and be of such size that they will be legible and not less than one millimeter in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:* Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:* **The author's name, title of paper, and figure number should be written in the lower left hand corner (outside the illustration proper) of the sheets on which the illustrations appear.** Captions should not be written on the illustrations, but typed on a separate paper of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.** If authors desire to alter a cut, they will be charged for the new cut.

TABLES: Each table should be typed on a separate sheet. Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES: **These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in this Journal. **Titles of papers and inclusive page numbers** of the references cited should be given. **All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number.

REPRINTS: A total of 50 reprints of each paper, without covers, are supplied free to the authors. Additional reprints will be supplied according to a prescribed schedule of charges. On request, covers can be supplied at cost.

Approximate charges for reprints may be calculated from the number of printed pages, obtained by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. × 11 in.) and making allowance for space occupied by line drawings and halftones (not inserts). The cost per page is tabulated at the back of the reprint request form sent with the galley.

Manuscripts should be addressed: *Canadian Journal of Zoology,*
National Research Council,
Ottawa, Canada.

Contents

Printed by EDMOND CLOUTIER, C.M.G., O.A., D.S.P., King's Printer, Ottawa, 1951.